unknown. In the present study we tested a hypothesis that cytoskeleton breakdown can lead to ED. Effects of cytochalasin D (CytD), a disrupter of F-actin were assayed on the key processes consecutively involved in electro-mechanical coupling: action potentials (APs), L-type Ca^{++} current (I_{CaL}), Ca^{++} transients, and cell contractions measured in rat ventricular cardiomyocytes at 37ºC. The measurements were performed by using both perforated and whole-cell patch-clamp, fluo-3 Ca^{++} indicator, and an edge movement detector. In CytD-treated cells relaxation kinetics was slower and contraction amplitude was reduced (about twice at 0.8 μM of CytD). In addition, CytD slowed decay of Ca^{++} transients (τ_{decay} = 47.3 ± 2.8 ms, n = 20; in control cells: 28.1 ± 1.3 ms, n = 28, mean ± SEM, $p < 0.01$). The rising phase of Ca^{++} transients was also significantly slower in CytD treated cells (τ_{rise} = 5.1 ± 0.6 ms, n = 17; in control cells: 3.6 ± 0.2 ms, n = 21, $p < 0.01$). The suppressive effect of CytD on cell contractions could not result only from the changes in kinetics of Ca^{++} transient since the amplitude of the transient (442 ± 51 nM, n = 8) and resting Ca^{++} level (89.4 ± 7.3 nM) changed insignificantly (in control cells: 424 ± 38 nM and 91.4 ± 10.8 nM, respectively, n = 13). Moreover, at higher CytD concentrations (from 4 to 40 μM), contraction was totally blocked, but APs, I_{CaL}, and amplitude of Ca^{++} transient did not change resulting in a complete ED. We conclude that integrity of F-actin-based cytoskeleton is an important factor for EC coupling. Particularly, myofibrils can not properly respond to Ca^{++} transient when cytoskeleton is damaged. We speculate that disruption of the cytoskeleton reported in ischemia can have an implication for ED phenomena as well as for insufficient cardiomyocyte contractility shown in heart failure.

4:30

811-3 Action Potential Voltage-Clamp Reveals Important Contribution of Ca^{2+} Current to Rate-Dependent Changes in Human Ventricular Action Potentials

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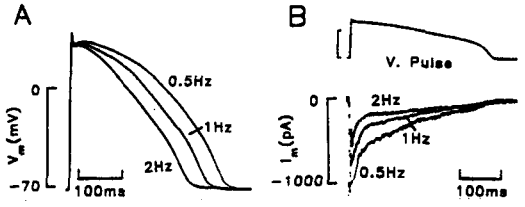
Rate-dependent changes in the action potential (AP) are known to be an important determinant of arrhythmias. The mechanism of AP abbreviation at rapid rates in human ventricular myocytes is unknown: both Ca^{2+} (I_{Ca}) and K^+ currents are potential candidates. To determine the role of I_{Ca} in human ventricular AP control, cells were studied at 36ºC with whole-cell patch clamp technique. Inactivation and recovery of I_{Ca} were biexponential (eg, τ's of 8 ± 1 and 86 ± 10 ms at +10 mV; recovery τ's of 18 ± 3 and 174 ± 25 ms at −80 mV). AP duration at 90% repolarization (APD_{90}) decreased by 34 ± 2% when frequency was increased from 0.5 to 2 Hz (Fig. A). I_{Ca} block (200 μM Cd^{2+}) shortened APD and strongly inhibited rate-dependent changes in APD_{90} (to 5 ± 2%, $p < 0.01$ vs control). AP clamp was performed using action potentials recorded from the same cell at 0.5 Hz, and demonstrated important rate-dependence of Cd-sensitive I_{Ca} (Fig. B): peak I_{Ca} was decreased by 34 ± 4% from 0.5 to 2 Hz (1034 ± 134 to 690 ± 45 pA, $n = 5$, $p < 0.01$).



The results indicate that I_{Ca} is an important determinant of plateau duration, and point to a strong contribution of rate-dependent I_{Ca} inactivation to frequency-related changes in APD, which may play a major role in governing re-entrant ventricular arrhythmias in man.

4:45

811-4 The "Reverse-Use Dependence" of the New Class III Antiarrhythmic Dofetilide Can Be Modulated by Pharmacologic Agents Influencing $[Ca^{2+}]_i$

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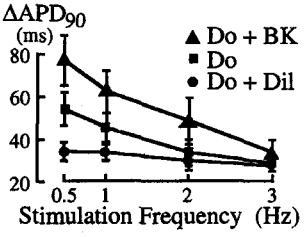
The "reverse-use dependence" characterizing the antiarrhythmic effect of most currently used class III agents render their antiarrhythmic profile clinically unfavourable. This phenomenon is thought to be related, at least in part, to the accumulation of the not completely deactivated I_{Ks} (slow component of the delayed rectifier potassium channel), whose magnitude depends on $[Ca^{2+}]_i$. We tested the hypothesis that this phenomenon could be modulated by pharmacologic agents influencing $[Ca^{2+}]_i$. APD at 90% repolarization (APD_{90}) was determined in guinea-pig right ventricular papillary muscles by mean of standard microelectrode technique at stimulation frequencies 0.5, 1, 2 and 3 Hz, first in control and then 30 min after 10 nM dofetilide (Do). Thereafter, either 10 μM diltiazem (n = 10) (Dil), or 0.1 μM Bay K 8644 (n = 11) (BK) was added to the bath solution in order to decrease or increase $[Ca^{2+}]_i$. Measurements were repeated 30 and 20 min after Dil and BK, respectively.



The prolongation of APD_{90} (ΔAPD_{90}) under Do, Do + Dil and Do + BK (mean ± SEM) is shown in the following picture. The APD_{90} prolongation by Do was markedly reduced at high frequencies. This was more pronounced in the presence of BK, but was prevented by Dil.

Conclusion: The "reverse-use dependent" effect of the class III agent dofetilide can be modulated by pharmacologic agents influencing $[Ca^{2+}]_i$.

812 Echo–Contrast Studies of Myocardial Blood Flow and Perfusion

Wednesday, March 19, 1997, 4:00 p.m.–5:00 p.m.
Anaheim Hilton and Towers, Pacific B

4:00

812-1 Myocardial Contrast Echocardiography Can Be Used to Quantify Intramyocardial Blood Volume: New Insights into Structural Mechanisms of Coronary Autoregulation

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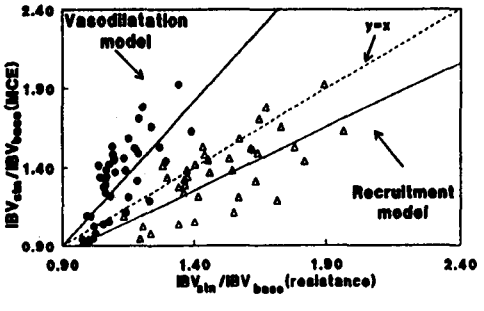
Changes in intramyocardial blood volume (IBV) mediate autoregulatory adaptations to coronary stenosis (STN). We tested the hypotheses that myocardial contrast echocardiography (MCE) could quantify changes in IBV in response to non-flow limiting coronary STN and that the relationship between coronary resistance- and MCE-derived IBV could yield insight into structural mechanisms of IBV change. The left anterior descending artery in 12 open chest dogs was instrumented with a flow probe, variable occluder, and intracoronary pressure catheter. MCE was performed using intra-aortic injection of Albunex during 3 to 5 non-flow-limiting coronary STN. IBV was derived using coronary resistance measurements applied to 2 theoretical models which assumed autoregulation to occur either via vasodilatation or microvascular recruitment. Flow was measured using radioactive microspheres, and MCE-determined IBV was calculated from microbubble transit rates.

At constant flow, MCE- and resistance- derived IBV were linearly related and increased with progressive STN (STN gradient 10–45 mmHg). MCE overestimated IBV derived by the vasodilatation model ($y = 1.84x - 0.76$, $p < 0.01$), and underestimated IBV calculated using the recruitment model ($y = 0.81x + 0.13$, $p < 0.01$) (Fig.).



MCE can quantify autoregulatory increases in IBV which maintain resting myocardial perfusion during coronary STN. Furthermore, these data suggest that both microvessel vasodilatation and recruitment simultaneously regulate IBV change. By detecting IBV heterogeneity, MCE may be a clinically useful technique for detecting and quantifying coronary disease under resting conditions.